

Dissection of Genomic Regions for Ion Homeostasis under Sodic Salt Stress in MAGIC Rice Population [†]

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Abstract: Salt tolerance mechanisms are regulated by balance in cell ionic concentrations such as K⁺, Na⁺, H⁺, Ca²⁺, and Mg²⁺. In this study, we examined major QTLs for the traits K⁺/Na⁺ homeostasis, shoot magnesium content (Mg²⁺), shoot calcium content (Ca²⁺), and shoot length. The QTLs for K⁺/Na⁺ homeostasis *Sod K/Na.1* are associated with three candidate genes: *LOC_Os02g48290*, *LOC_Os02g48340*, and *LOC_Os02g48350*, and *Sod_Ca.1* is associated with the gene *LOC_Os08g15020*. Three significant candidate gene haplotypes for shoot length, *Sod_SL.1* (*LOC_Os10g36690*), sodium content *Sod_Na.1* (*LOC_Os01g41770*), and magnesium content *Sod_Mg.1* (*LOC_Os10g31040*) were identified. The identified candidate genes encode dehydration response proteins, leucine rich repeat proteins, citrate transporter proteins, and diacylglycerol O-acyltransferase (DGATs), and play a key role in salt and abiotic stress tolerance. The identified novel QTLs and potential candidate genes could be used for functional characterization to help further supplement our understanding of the genetic makeup of sodicity stress tolerance in rice.

Keywords: GWAS; MAGIC; GBS; sodicity; rice; aluminium tolerance; QTL; salt stress



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1. Introduction

Rice is an important staple food for more than 3.5 billion people in the world and is cultivated in 114 countries [1]. Exploding population with urbanization are not only threats to food security, but also affect global climate change by increasing temperature and decreasing cultivable lands. The effect of climate change on the agriculture sector is creating massive emerging problems such as biotic and abiotic stresses. Among the abiotic stresses, salt stress (salinity and sodicity) is the most important environmental factor hampering crop productivity. More than 6% of the world's (900 Mha) soil is facing intrusion by salt [1]. During salt stress, crops experience ion imbalances, ion toxicity, and reduced water potential, which affects the normal plant metabolism and crop yield [2]. Many researchers have reported the effect of sodic soils on growth and development in rice [3]. Excess Na⁺ accumulation creates ionic stress in the aerial parts of plants since Na⁺ interferes with plant physiology, including imbalances in the homeostasis of other ions such as K⁺, Ca²⁺, and Mg²⁺. Hence, high cytosolic K⁺/Na⁺ ratios become a key salt tolerance trait. Several QTLs were identified for salinity tolerance in rice [4,5]. A major QTL, *Saltol*, was mapped on chromosome 1 and was introgressed into mega rice varieties [6–8]. However, less information is available on the molecular basis for sodicity tolerance. In this study, we intended to find the effect of sodicity on the *indica* MAGIC population and to identify the novel QTLs for sodicity tolerance in rice at the seedling stage.

2. Materials and Methods

The plant material consisted of 391 rice MAGIC lines developed from an intercrossing of eight founder lines at the International Rice Research Institute, Philippines [9]. These lines were evaluated under control and alkaline condition at ICAR-CSSRI, Karnal. The seeds of rice lines were sown in soil contained within a tray. The desired pH of the soil (pH ~9.7–9.8) was created on 14 DAS using sodium bicarbonate (NaHCO_3) and sodium carbonate (Na_2CO_3) solution. The data on the morphological traits, shoot length, and tissue samples were taken to measure physiological parameters such as shoot Na^+ , K^+ , Ca^{2+} , and Mg^{2+} content (ppm) 14 days after stress, and the K^+/Na^+ ratio was calculated. Genotyping of founder and MAGIC lines was done by sequencing using the Illumina Hi Seq method. The processed credible 27,041 SNP sites were then used for marker-trait association studies (MTA).

MTAs were identified using Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) implemented using the genomic association and prediction integrated tool (GAPIT). The SNP positions of genes associated with traits of interest were considered as candidate genes. The haplotype analyses for identified candidate genes were conducted by CandiHap V2 (<https://github.com/xukaili/CandiHap>, accessed on 20 December 2020). Visualization of the violin plot was done using the ggplot2 package in R programme.

3. Results and Discussion

Genotype by sequencing (GBS) data of 391 *indica* MAGIC rice lines were used for association for morph-physiological traits such as shoot length, shoot Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and tissue K^+/Na^+ ratio. The eight QTLs distributed on chromosomes 1, 2, 4, 8, and 10 that were responsible for sodicity tolerances at the seedling stage are presented in Table 1.

The QTL *Sod_SL.1* was responsible for shoot length exhibiting 9.01% phenotypic variance. The peak SNP (Position—19621750 on Chromosome 10) of *Sod_SL.1* QTL was lying in the genic region of *LOC_Os10g36690* and encoded a dehydration response protein to have a significant role under salt stress [10]. The given gene is characterized as a member of the dehydration responsive element-binding (DREB) super family of genes, and over expression of the *OsDREB1F* gene is confirmed to enhance salt tolerance in rice [11]. Three QTLs *Sod_Na.1*, *Sod_Na.2*, and *Sod_Na.3* for shoot sodium content were displaying 3.46, 6.22, and 5.23% phenotypic variance, respectively. The peak SNPs position-23642384 on Chromosome 1 was lying in the genic region of *LOC_Os01g41770* encoding leucine-rich repeat protein. The role of the leucine-rich repeat receptor-like kinases gene (*OsSTLK*) in response to salt tolerance was reported in rice [11,12]. Over expression of *OsSTLK* exhibited reduced malondialdehyde (MDA) content, electrolyte leakage, and reactive oxygen species (ROS) under salt stress conditions. The only QTL (*Sod_K.1*) was detected with an LOD of 3.43 for shoot potassium content lying in the genic region of *LOC_Os02g32814*. The effects of both sodicity and aluminium became evident at pH~9.0 and waning at pH > 9.2 [12,13]. Hence, the role of genes responsible for metal toxicity under sodic condition cannot be ignored. QTLs for shoot calcium content (*Sod_Ca.1*) and magnesium content (*Sod_Mg.1*) exhibited 9.8 and 20.65 phenotypic variance. The peak SNP responsible for calcium content present on the gene *LOC_Os08g15020* encoding the MYB family transcription factor plays a key regulatory role under drought and salinity [14]. Further, an independent study has also functionally characterized the positive role of the MYB gene under salt stress in rice [15]. The gene in this QTL is a probable MYB, which has the capacity to stimulate plant growth through calcium signaling under salt stress [16]. The peak SNP associated with QTL *Sod_Mg.1* was lying in the genic region of *LOC_Os10g31040*, responsible for citrate transporter protein. In the QTL *Sod_K/Na.1* responsible for K^+/Na^+ homeostasis, we observed six peak SNP markers tracked between the 289 kb region from 29.313 to 29.602 Mb. The peak SNP positions in *Sod_K/Na.1* were lying in the regions of *LOC_Os02g48290*, *LOC_Os02g48340*, and *LOC_Os02g48350* (*OsDGAT*), which encodes thioredoxin reductase, RNA recognition motif containing (RRM) protein and diacylglycerol O-acyltransferase

(DGATs), respectively. The role of thioredoxin reductase in seedling development, photosynthetic metabolism, and plant growth in response to varying light conditions and starch degradation in guard and mesophyll cells under osmotic stress was reported earlier [17–19]. RNA binding proteins (RBP) are able to increase the yeast Na^+ -tolerance, *Beta vulgaris*. Salt-tolerant (BvSATO) genes BvSATO1, BvSATO2, BvSATO4, and BvSATO6 were RRM containing proteins involved in RNA metabolism, developmental processes, and played an important role in salt tolerance [20]. The co-expression of *OsTCP19* and *LOC_Os02g48350* (*OsDGAT*) regulating the triacylglycerol biosynthesis by modulating ABI4-mediated pathways under salt and drought stress conditions was reported in rice [21]. The haplotype analyses for candidate genes linked to seedling stage sodicity tolerance suggested that three genes (*LOC_Os10g36690*, *LOC_Os01g41770*, and *LOC_Os10g31040*) show significant haplotypes associated with QTLs Sod_SL.1, Sod_Na.1, and Sod_Mg.1, respectively (Figure 1). Identified QTLs associated with sodicity tolerant and respective candidate genes responsible for abiotic stress can be further reinvestigated to confirm their role in salt tolerance in rice.

Table 1. Associated QTLs with SNP position and probable candidate genes for sodicity tolerance in MAGIC population.

Sl. No	Trait	QTL	Chromosome	Position	p Value	LOD	Locus ID	Gene Annotation
1	Shoot length	<i>Sod_SL.1</i>	10	19621750	1.50×10^{-5}	4.82	<i>LOC_Os10g36690</i>	Dehydration response related protein, putative, expressed
2	Na	<i>Sod_Na.1</i>	1	23642384	1.54×10^{-5}	4.81	<i>LOC_Os01g41770</i>	Leucine rich repeat protein, putative, expressed
3		<i>Sod_Na.2</i>	4	11638572	7.35×10^{-5}	4.13	<i>LOC_Os04g20749</i>	Expressed protein
4		<i>Sod_Na.3</i>	8	4773918	2.38×10^{-6}	5.62		
5	K	<i>Sod_K.1</i>	2	19479355	0.000375	3.43	<i>LOC_Os02g32814</i>	Heavy metal-associated domain containing protein, expressed
6	Ca	<i>Sod_Ca.1</i>	8	9069848	1.11×10^{-5}	4.95	<i>LOC_Os08g15020</i>	MYB family transcription factor, putative, expressed
7	Mg	<i>Sod_Mg.1</i>	10	16228910	3.41×10^{-7}	6.47	<i>LOC_Os10g31040</i>	Citrate transporter protein, putative, expressed
8	K/Na	<i>Sod_K/Na.1</i>	2	29313685	1.60×10^{-8}	7.80	<i>LOC_Os02g48290</i>	Thioredoxin reductase 2, putative, expressed RNA recognition motif containing protein, putative, expressed Diacylglycerol O-acyltransferase, putative, expressed
9			2	29550035	2.60×10^{-5}	4.58		
10			2	29596887	1.22×10^{-5}	4.91		
11			2	29602570	1.22×10^{-5}	4.91		
12			2	29602596	1.22×10^{-5}	4.91		
13			2	29602600	1.22×10^{-5}	4.91	<i>LOC_Os02g48350</i>	

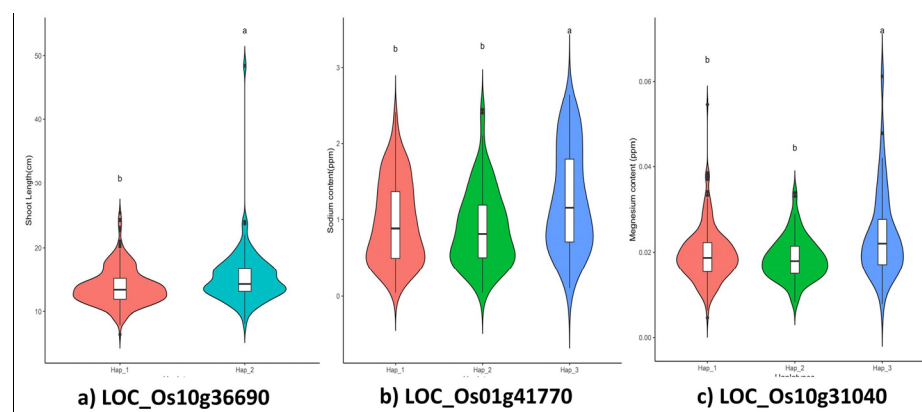


Figure 1. Violin plot indicating the significant haplotypes for candidate genes associated with sodic tolerance QTL; (a) shoot length; (b) shoot sodium content; (c) magnesium content among *indica* MAGIC rice lines (X-axis = Haplotypes; Y-axis = Trait value). Each box plot within the violin plot represents minimum, lower quartile, median, upper quartile, and maximum values. Different letters above the violin plots indicate statistically significant differences for the respective haplotypes, at a significance level of $p < 0.05$ (Duncan test).

4. Conclusions

In the present study, we identified thirteen SNPs associated with eight QTL regions responsible for sodicity tolerance. We detected major QTLs for the traits K^+/Na^+ homeostasis, shoot magnesium content (Mg^{2+}), shoot calcium (Ca^{2+}), and shoot length, explaining about 32.02%, 20.65%, 9.83%, and 9.01% phenotypic variance, respectively. We found three significant candidate gene haplotypes associated with the QTLs for shoot length (LOC_Os10g36690), shoot sodium content (LOC_Os01g41770), and shoot magnesium content *Sod_Mg.1* (LOC_Os10g31040). The identified QTL regions and candidate genes play significant roles in sodic stress tolerance, which can be further investigated.

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